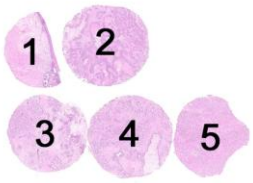
Assessment Run B13 2012

Estrogen Receptor (ER)

Material

The slide to be stained for ER comprised the following five tissues:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80 - 90 %	Moderate to strong
2.	Breast ductal carcinoma	Negative	Negative
3.	Breast ductal carcinoma	60 - 80 %	Weak to moderate
4.	Breast ductal carcinoma	40 - 60 %	Weak to moderate
5.	Breast ductal carcinoma	80 - 100 %	Moderate to strong



All tissues were fixed in 10% neutral buffered formalin for 24 – 48 hours and processed according to Yaziji et al. (1).

*ER-status and staining pattern as characterized by NordiQC reference laboratories using the rmAb clone SP1.

Criteria for assessing an ER staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of most columnar and squamous epithelial cells as well as stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- An at least weak to moderate distinct nuclear staining reaction of the appropriate proportion of the neoplastic cells in the breast ductal carcinomas no. 3 and 4 and correspondingly an at least moderate to strong reaction in no. 5.
- No nuclear staining reaction in the neoplastic cells in the breast carcinoma no. 2 and no more than a weak cytoplasmic staining reaction in cells with a strong nuclear staining reaction.

A staining was classified as good if ≥ 10 % of the neoplastic cells in the breast ductal carcinomas no. 3, 4 & 5 showed an at least weak nuclear staining reaction but less than the reference ranges.

A staining was assessed as borderline if ≥ 1 % and < 10 % of the neoplastic cells showed a nuclear staining reaction in one or more of the breast ductal carcinomas no. 3, 4 & 5.

A staining was assessed as poor if < 1% of the neoplastic cells showed a nuclear staining reaction in one or more of the breast ductal carcinomas no. 3, 4 and 5 or a false positive nuclear staining reaction was seen in the breast ductal carcinoma no. 2.

241 laboratories participated in this assessment. 85 % achieved a sufficient (optimal or good) mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for ER, run B13**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1D5**	10 2 1	Dako Immunologic Immunovision	2	7	1	3	69 %	100 %
mAb clone **6F11**	30 3 1	Leica/Novocastra Vector Monosan	11	15	4	4	76 %	86 %
mAb clones **1D5 + 6F11**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb **EP1**	4 1	Dako Epitomics	3	1	1	0	80 %	100 %
rmAb **SP1**	26 4 4 3 2	Thermo/NeoMarkers Dako* Spring Cell Marque Immunologic	18	11	4	7	73 %	82 %

	1	Master Diagnostica						
Ready-To-Use Abs:								
mAb clone **1D5 IR/IS657**	16	Dako	3	7	1	5	63 %	69 %
mAb clone **6F11 PA0151**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clones **1D5 + 6F11 PM308**	1	Biocare	1	0	0	0	-	-
mAb clones **1D5 + ER-2-123 K4071/SK301**	4	Dako	1	1	1	1	-	-
rmAb clone **EP1 IR/IS084**	21	Dako	17	2	2	0	90 %	90 %
rmAb clone **EP1 ZA-0102**	1	Zhongshan	0	0	1	0	-	-
rmAb clone **SP1 790-4324/25**	97	Ventana	80	16	0	1	99 %	99 %
rmAb clone **SP1 IR/IS151***	6	Dako	2	3	1	0	83 %	-
rmAb clone **SP1 RM-9101-R7**	1	Thermo/NeoMarkers	1	0	0	0	-	-
Total	241		139	65	16	21	-	
Proportion			58%	27 %	7 %	8 %	85 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below
* Product has been discontinued by the vendor

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **1D5**: The two protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (1/5)* or TRS pH 9 (Dako) (1/3) as the retrieval buffer.
The mAb was diluted 1:100. Using these protocol settings 2 out of 2 (100 %) laboratories produced an optimal staining result.
* (number of optimal results/number of laboratories using this reagent)

mAb clone **6F11:** The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (3/3), TRS pH 9 (Dako) (1/1), TRS pH 6.1 (Dako) (1/2), Cell Conditioning 1 (CC1; BenchMark, Ventana) (1/3), Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (2/12) or Tris-EDTA/EGTA pH 9 (3/6) as the retrieval buffer.
The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 19 out of 22 (86 %) laboratories produced a sufficient staining result (optimal or good).

rmAb **EP1:** The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (1/2), TRS pH 9 (Dako) (1/1) or Tris-EDTA/EGTA pH 9 (1/1) as the retrieval buffer.
The rmAb was diluted in the range of 1:30-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced an optimal staining result.

rmAb clone **SP1**: The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (TRS pH 9; Dako) (1/5), TRS pH 9 (Dako) (1/4), CC1 (BenchMark, Ventana) (4/10), BERS 1 (Bond, Leica) (1/1), BERS 2 (Bond, Leica) (3/3), Borg Decloaker pH 9.5 (Biocare) (1/1), Tris-EDTA/EGTA pH 9 (3/6), EDTA/EGTA pH 8 (2/2) or Citrate pH 6.7 (2/2) as the retrieval buffer.
The rmAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 out of 34 (82 %) laboratories produced a sufficient staining result (optimal or good).

Ready-To-Use Abs
mAb clone **1D5** (prod. no. IR/IS657, Dako): The protocols giving an optimal result were typically based on HIER in PT-Link (heating time for 10-30 min at 96°C- 99°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20-30 min in the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as the detection system. Using these protocol settings 9 out of 13 (69 %) laboratories produced a sufficient staining result.

mAb clones **1D5 + 6F11** (prod. no. PM308, Biocare): The protocol giving an optimal staining result was based on HIER in a Pressure Cooker using Diva Decloaker pH 6.2 as HIER buffer, an incubation time of 45 min in the primary Ab and MACH4 Universal HRP Polymer as the detection system.

mAb clones **1D5 + ER-2-123** (prod. no K4071/SK310, Dako (pharmDx™ kit): The protocol giving an optimal result was based on HIER using Epitope Retrieval Solution (K4071/SK310) in a pressure cooker, an incubation time of 30 min in the primary Ab and K4071/SK310 (pharmDx™ kit) as the detection system. Using these protocol settings 2 out of 4 (50 %) laboratories produced a sufficient staining result.

rmAb clone **EP1** (prod. no. IR/IS084, Dako): The protocols giving an optimal result were typically based on HIER in PT-Link (heating time for 10-20 min at 95°C- 97°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20-30 min in the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as the detection system. Using these protocol settings 19 out of 20 (95 %) laboratories produced a sufficient staining result.

rmAb clone **SP1** (prod.no 790-4324/25, Ventana): The protocols giving an optimal result were typically based on HIER using mild or standard CC1, an incubation time of 8-40 min in the primary Ab and iView (760-91), UltraView (760-500) or OptiView (760-700) as the detection system. Using these protocol settings 96 out of 97 (99 %) laboratories produced a sufficient staining result.

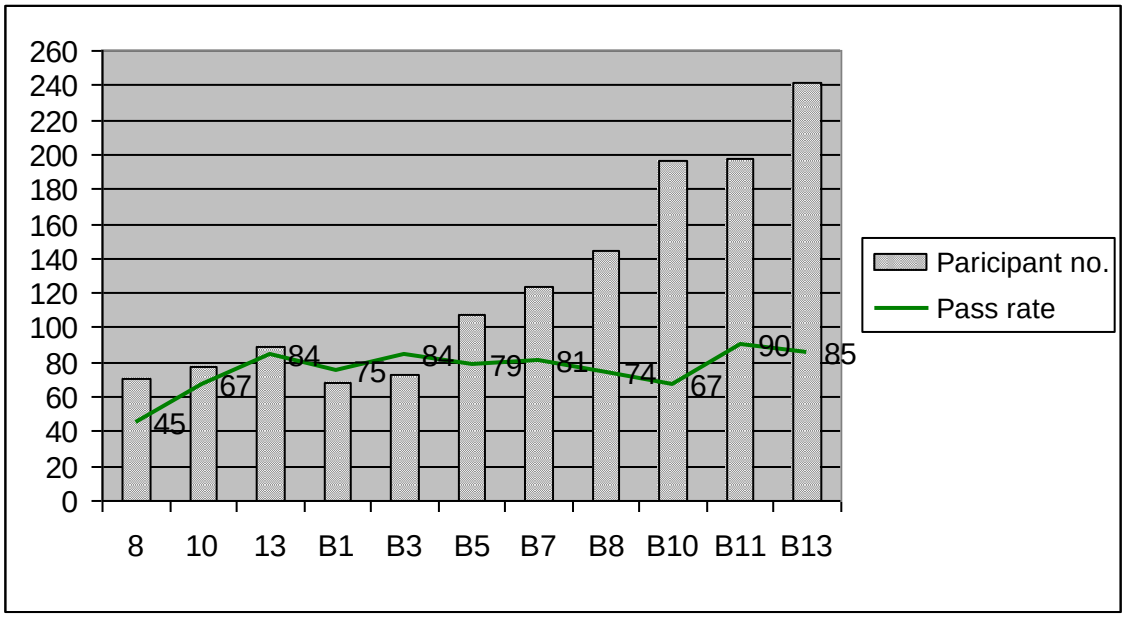
The most frequent causes of insufficient staining results were:

- Too low concentration of the primary antibody
- Insufficient HIER (use of citrate pH 6.0 and/or too short efficient heating time)

In this assessment the prevalent feature of an insufficient staining result was a generally too weak or false negative staining, especially seen in the breast ductal carcinomas no. 3 & 4, in which an at least a weak nuclear staining reaction of 40-80% of the neoplastic cells was expected. This pattern was seen in 31 out of 37 of the insufficient results (84 %) and was typically caused by insufficient HIER and/or a too low concentration of the primary Ab. In the remaining insufficient results a false positive staining reaction and/or poor signal-to-noise ratio was seen and was most critically characterized by a weak but consistent nuclear staining reaction in the ER negative breast ductal carcinoma no. 5 as well as other cells like lymphocytes and endothelial cells. The false positive staining reaction was typically caused by a too high concentration of the primary Ab (mainly the mAb clone 6F11 applied in the range of 1:20-40) in combination with an immunhistochemical protocol with a high sensitivity based on HIER in an alkaline buffer and a 3-step polymer based detection system. However it has to be emphasized that identical protocol settings (causing the false positive staining reaction) in most cases gave optimal staining results. As observed in the previous ER assessments, all the 3 most widely used Abs for ER, the mAb clones 1D5 and 6F11 and the rmAb clone SP1 could all be used to obtain an optimal staining result, but clone 1D5 still gave the largest proportion of insufficient stains. In this assessment the newly launched rmAb clone EP1 from Dako and Epitomics was applied for the first time and gave a high proportion of sufficient and optimal staining results.

This was the 11th assessment of ER in NordiQC and a slight decrease in proportion of sufficient results was seen compared to run B11, as seen in table 2.

Table 2. **Proportion of sufficient results for ER in the NordiQC assessments performed**



Despite a slight decrease in the proportion of sufficient results compared to the previous run B11 was seen, the overall pass rate still was maintained at a high level, superior to the pass rates seen in runs B8 and B10. A slight difference regarding the pass rates was observed for the laboratories participating in the ER assessment for the first time compared to the laboratories also participating in the latest assessment run B13, 2011: For the laboratories participating for the first time the pass rate was 77 % (48 out of 62 laboratories), whereas the pass rate was 88 % (156 out of 179 laboratories) for the laboratories participating in both runs.

As seen in the previous run for ER, an important factor to obtain and maintain the high pass rates was the impact from the extended use of properly calibrated and commercially available Ready-To-Use (RTU) systems for ER instead of in-house calibrated assays. E.g., in the current run the RTU systems based on the rmAb clone SP1 from Ventana and the rmAb clone EP1 from Dako were used by 118 out of the 241 participating laboratories (49 %) and grouped together a pass rate of 98 % was obtained (115 out of 118 laboratories). Using the same clones as concentrates and applied with an in-house validated assay the pass rate was only 73 % (32 out of 44 laboratories).

As shown in the previous runs, uterine cervix was an appropriate positive control for the ER staining: In the optimal protocols virtually all the epithelial cells throughout the layers of the squamous epithelium and in the glands showed a moderate to strong and distinct nuclear staining reaction. In the stromal compartment a moderate to strong nuclear staining reaction in most cells was seen and only endothelial and lymphatic cells were negative. If the staining reaction in the epithelial cells of the uterine cervix was significantly reduced, a too weak staining reaction was also seen in the breast carcinomas no. 3 & 4. In these tumours the normal epithelial cells consistently were demonstrated, emphasizing that normal breast tissue is not reliable as an internal positive control for a correct calibrated immunohistochemical assay for ER.

Conclusion

The mAb clones 1D5, 6F11 and the rmAb clones EP1 and SP1 could all be used to obtain a sufficient staining result. The rmAb clones EP1 and SP1 provided a higher proportion of optimal results.
In this assessment the widely used RTU systems for ER based on the rmAb clone SP1 (Ventana) and EP1 (Dako) gave higher pass rates for the demonstration of ER than the in-house validated assays.
HIER is mandatory, preferable in an alkaline buffer.
Uterine cervix is an appropriate control for ER: Virtually all the epithelial cells and most stromal cells must show a strong distinct nuclear staining reaction with a minimal cytoplasmic reaction.

1. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20. PubMed PMID: 18931614.

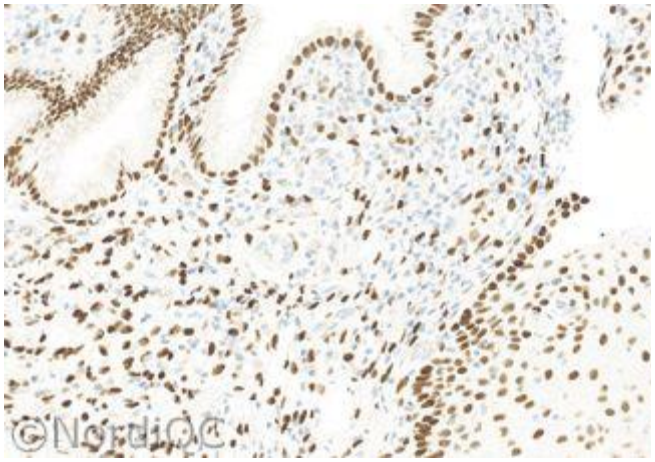


Fig 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 optimally calibrated and with HIER in an alkaline buffer. Virtually all the squamous and columnar epithelial cells show a moderate to strong, distinct nuclear staining reaction. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative.

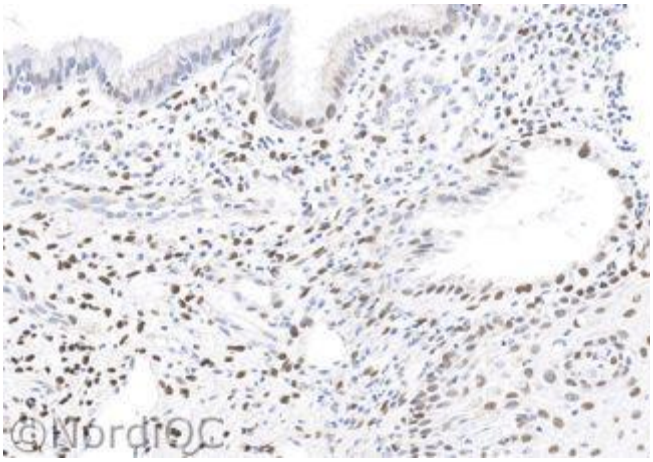


Fig 1b
Insufficient ER staining of the uterine cervix, same field as in Fig. 1a. The proportion and intensity of the staining reaction in the squamous and columnar epithelial cells is reduced. Also compare with Figs. 2b - 4b – same protocol. The protocol was based on the rmAb clone SP1 applied with protocol settings giving a too low sensitivity – most likely a combination of insufficient HIER and a too dilute primary Ab.

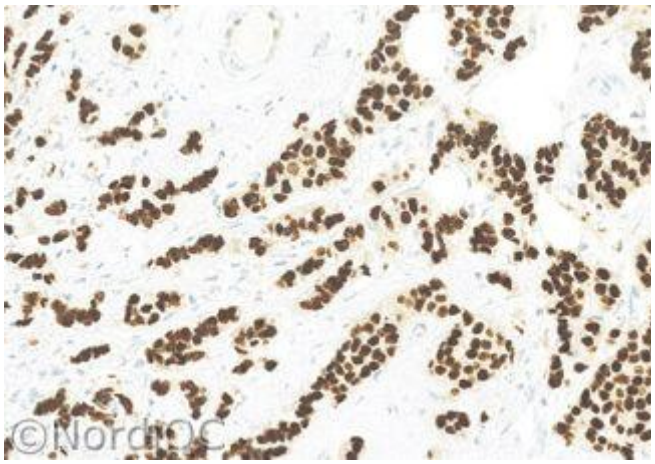


Fig 2a
Optimal ER staining of the breast ductal carcinoma no. 5 with 80 – 100 % cells positive. Virtually all the nuclei of the neoplastic cells show a strong, distinct nuclear staining reaction with only a weak cytoplasmic staining reaction. No background staining is seen.
Same protocol as in Fig. 1a.

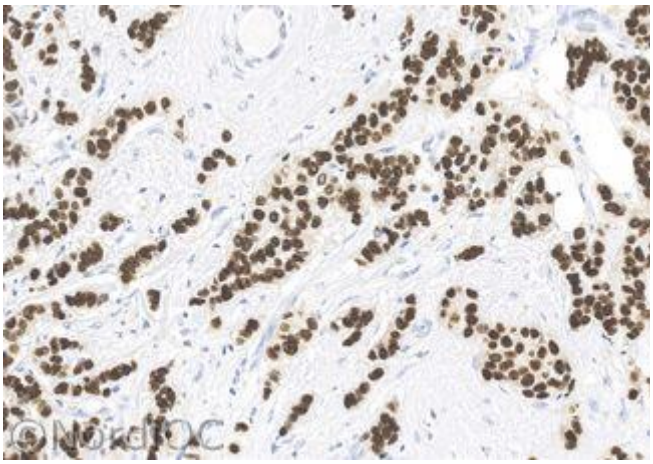


Fig 2b
ER staining of the breast ductal carcinoma no. 5 with 80 – 100 % cells positive using the same insufficient protocol as in Fig. 1b – same field as in Fig. 2a. Virtually all the neoplastic cells are demonstrated, but also compare with Figs. 2b - 4b – same protocol.

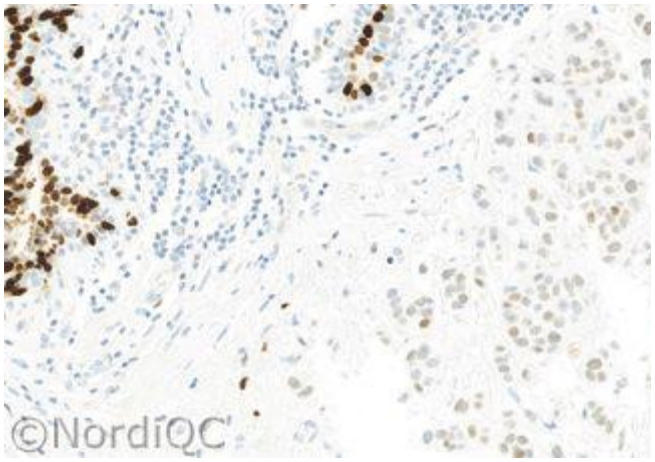


Fig 3a
Optimal ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

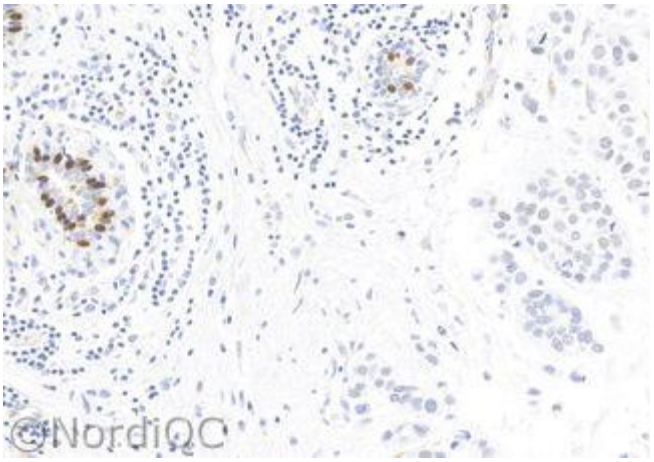


Fig 3b
Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. Only dispersed neoplastic cells show an equivocal staining reaction.

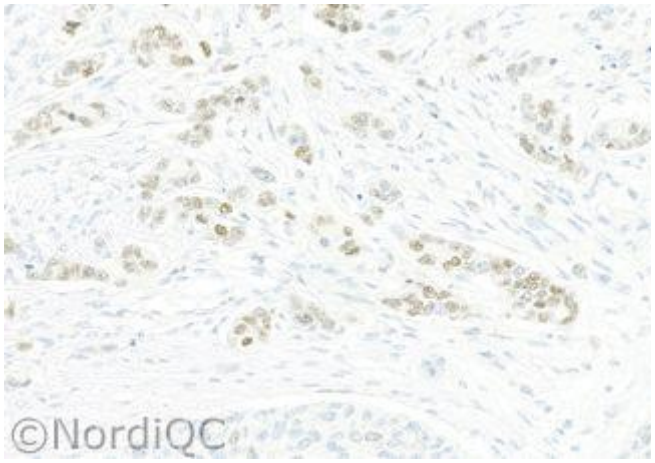


Fig 4a
Optimal ER staining of the breast ductal carcinoma no. 4 with 40 – 60 % cell positive. A weak to moderate staining reaction is seen in the appropriate proportion of the neoplastic cells and no background staining is seen. Same protocol as in Figs. 1a – 3a.

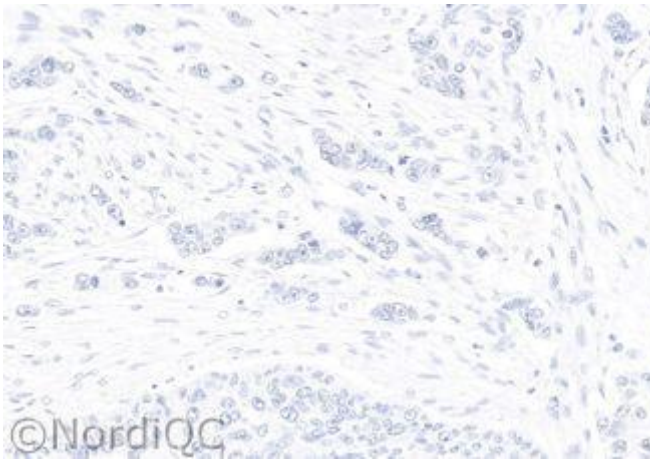


Fig 4b
Insufficient ER staining of the breast ductal carcinoma no. 4 with 40 – 60 % cells positive using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a. A false negative staining reaction is seen.

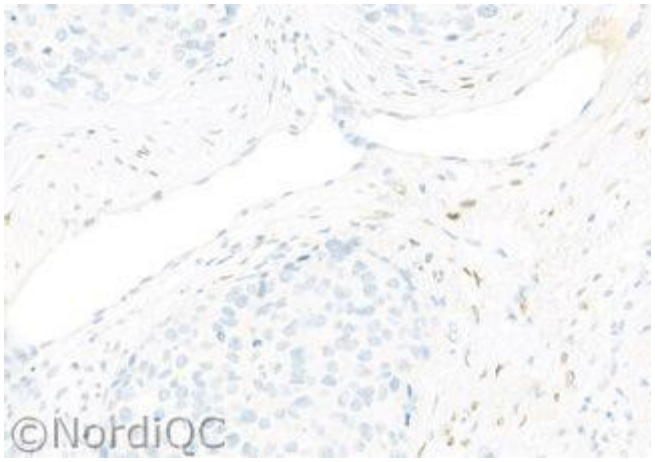


Fig 5a
Optimal ER staining of the breast ductal carcinoma no. 2. No nuclear staining reaction is seen in the neoplastic cells and only scattered stromal cells show a distinct nuclear staining reaction.
Same protocol as in Figs. 1a – 4a
Compare the optimal result with the aberrant and insufficient staining result in Fig. 5b.

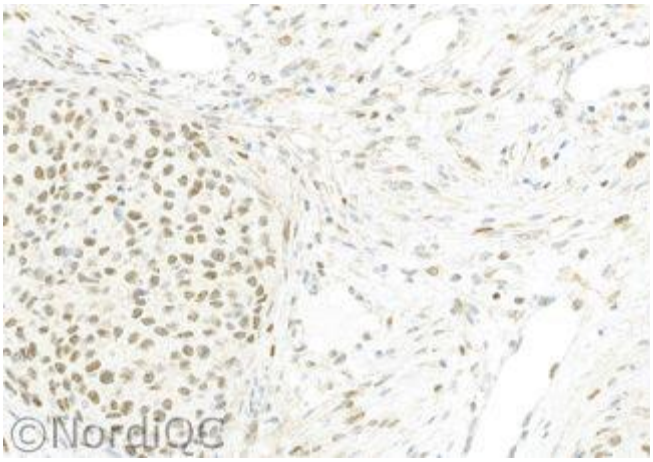


Fig 5b
Insufficient ER staining of the breast ductal carcinoma no 2. A moderate nuclear staining reaction is seen in the vast majority of the neoplastic cells. However also note the aberrant nuclear staining reaction in virtually all cells as e.g. the endothelial cells and in general a reduced signal-to-noise ratio is observed. The insufficient result most likely was caused by a combination of a too concentrated format of the primary Ab and a protocol with a very high sensitivity (HIER in an alkaline buffer and a 3-step polymer based detection system).

SN/MV/LE 10-7-2012